Characterisation of Project Constructs

Constructs A to E:

Day 1: For each measurement, 3 candidate colonies (in *E. coli* strain JW0336) containing the construct of interest were inoculated separately in LB medium supplemented with 50µg/ml kanamycin overnight at 37ºC with 250rpm shaking.

Day 2: 500µl of overnight culture is extracted and washed with 0.85% NaCl solution. The sample OD_{600} after wash is measured and hereafter diluted to 0.05 A.U. with LB to a total volume of 500ml. The diluted culture is incubated in 96-well Deep Well Plate, sealed with paraffin and inoculated at 37ºC with 250rpm shaking. OD_{600} was monitored and when most culture in the wells reached OD_{600} from 0.4-0.6, 200 µl of the culture was removed and washed with 0.85% NaCl and is then transferred to a 96-well microtiter plate for measurement. Experiments were repeated for three times on different days as technical triplicates.

Fluorescence measurement was performed using the EnVision Multilabel Reader with the following conditions:

- Absorbance: Photometric 595nm,
- Excitation: 485nm FITC,
- Emission: 535nm FITC,
- Mirror module: FITC (403) on top.

Tristable Switch Prototype:

Day 1: For each measurement, 3 candidate colonies (in *E. coli* strain JW0336) containing the construct of interest were inoculated separately in LB medium supplemented with 34µg/ml chloramphenicol overnight at 37°C with 250rpm shaking

Day 2:

Step 1: 500µl of overnight culture is extracted and washed with 0.85% NaCl solution.

Step 2: The sample OD_{600} is measured and hereafter diluted to 0.05 A.U. with LB and one of the three inducers (DAPG, aTc and IPTG) at working concentration of 25µM, 0.35µM and 20mM, respectively, in a total volume of 500ml. The diluted culture is incubated in 96-Well Deep Well Plate, sealed with paraffin and inoculated at 37°C with 250rpm shaking.

Step 3: OD_{600} was monitored and when most culture in the wells reached OD_{600} from 0.4-0.6, 200 µl of the culture was removed and washed with 0.85% NaCl and is then transferred to a 96-well microtiter plate for measurement.

Step 4: Repeat Step 2 and 3 twice with the remaining two inducers and in the end with the first inducer to complete one loop of state change.

There are in total six experimental set-ups, accounting for all six permutations of inducer input sequence. Experiments were repeated for three times on different days as technical triplicates.

Fluorescence measurement was performed using the EnVision Multilabel Reader with the following conditions:

- GFP:
  - Absorbance: Photometric 595nm,
  - Excitation: 485nm FITC,
  - Emission: 535nm FITC,
  - Mirror module: FITC (403) on top.

- RFP:
  - Absorbance: Photometric 595nm,
  - Excitation: 531nm TAMRA Acyclo Prime SNP excitation,
  - Emission: 579nm TAMRA FP P-pol 579,
  - Mirror module: BODIPY TMR FP.

- mTagBFP:
  - Absorbance: Photometric 595nm,
  - Excitation: 399 nm DAPI,
  - Emission: 456 nm DAPI,
  - Mirror module: CFP/YFP (428) bottom.